

# THE LOCI OF CONTACT CHEMORECEPTORS INVOLVED IN FEEDING REACTIONS IN CERTAIN LEPIDOPTERA<sup>1</sup>

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In our review (1949) of literature on the loci of contact chemoreceptors of insects we listed 10 species of butterflies which had been shown experimentally to have contact chemoreceptors on the tarsi. It was assumed that these insects also had receptors on the mouth-parts or in the mouth, but this had not been shown experimentally. Up to that time, four species of moths had been tested for possible possession of tarsal chemoreceptors and found not to possess them. At that time, we tested three species of butterflies, two of which had been shown previously to have tarsal chemoreceptors, located these more exactly and found that the terminal part of the proboscis in each is sensitive to chemical stimulation by solutions. The antennae and palpi were found not to be loci of contact chemoreceptors sensitive to sucrose solutions. The contact chemoreceptors on the tarsi and proboscis are involved in feeding reactions, and these should probably be so designated to differentiate them from chemoreceptors possibly involved in mating (Roth and Willis, 1952) or oviposition (Dethier, 1947).

As an extension of the earlier work, we have located the trophic contact chemoreceptors of four species of butterflies, only one of which has been tested previously, and two species of moths, neither of which has been tested previously.

## MATERIALS AND METHODS

The following species of butterflies were studied. The scientific names are those given by Klots (1951).

### Family: Satyridae

*Cercyonis pegala*—The Wood Nymph or Grayling

subsp. *alope* and *nephele*

20 individuals, captured in the field

*Lethe corydice*—The Eyed Brown

3 individuals, captured in the field

### Family: Nymphalidae

*Speyeria cybele*—The Great Spangled Fritillary

2 individuals, captured in the field

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*Limenitis arthemis*—The White Admiral  
2 individuals, captured in the field

The following species of moths were tested. The scientific names are those given by Borror and Delong (1954).

Family: Amatiidae (Syntomidae, Ctenuchidae, Euchromiidae)  
*Ctenucha virginica*  
22 individuals, captured in the field  
*Scepsis fulvicollis*—The Yellow-collared Scape Moth  
15 individuals, captured in the field

In the first and last two species, these were approximately half males and half females. *Cercyonis pegala* was found by Anderson (1932) to have tarsal chemoreceptors; the others have not previously been tested. Reporting results obtained with the small number of individuals of the last three butterflies in the list is justified only because the responses were so clear-cut and so similar to those of the other related species. The moths are day-flying and feed on nectar, and thus differ in habits from the usual moths. They have well developed proboscides.

The butterflies were immobilized for testing as described by Minnich (1921, 1922a, 1922b) by clamping the wings together with spring clothes-pins. The moths were mounted by fixing the dorsum of the prothorax and the wings to wax blocks on the ends of glass rods, as described by Frings (1947). Thus fixed, the animals could easily be handled and observed.

The contact chemoreceptors were located as described previously (Frings and Frings, 1949). Series of tests of variable duration and number were made each day on the fixed animals. Before each series the insects were given all the water they would drink. These butterflies and moths proved to be avid drinkers, and often kept the proboscis extended for more than 10 minutes. A 1 *M* sucrose solution in water was used for most of the tests, and contact of this with the receptors elicited uncoiling of the proboscis and feeding if allowed. The proboscis response could thus be used as the index of reception. Temperatures were uncontrolled and varied from 18°–25° C.

For general location of the receptors, the part to be tested was either immersed in the sucrose solution in a small dish or the solution was brought to the part on small artists' brushes. Each test with the solution was preceded by one or more control tests with water. If at any time the insects responded to water, they were allowed to drink until sated, were thereupon put aside for a time, and were retested later.

For more specific location of the receptors, two methods were used. Local stimulation of suspected parts with droplets of sucrose solution on finely drawn, glass microneedles was used with uninjured insects. These tests were also preceded by control tests with water. Operations on the animals for removal of suspected or known loci and later testing were also performed, with paired controls similarly treated except for the removal of parts. Generally, the insects were unanaesthetized for these manipulations. The tests were carried out under a binocular dissecting microscope at up to 30 × magnification.

For microscopic examination of possible end-organs, structures were removed to 70% ethyl alcohol, transferred to 95% alcohol and mounted on slides in Diaphane.

## RESULTS

In these butterflies, the rudimentary fore-legs characteristic of the Nymphaloids, the palpi, and the antennae seem to lack receptors for sucrose. The antennae are quite sensitive to water vapor if the animals are thirsty. Receptors are present on the ventral sides only of the tarsi of the mesothoracic and metathoracic legs and on the distal portion of the proboscis, extending about  $\frac{1}{4}$  of the length of the proboscis from the tip. With *C. pegala* and *L. carydice*, but not with the other two species, local stimulation techniques showed that the three distal segments of the mesothoracic and metathoracic tarsi have receptors and the basal segment does not. The second segment of the mesothoracic tarsus in *C. pegala* definitely has the receptors along its entire length, while that of *L. carydice* has them only at the distal end. Tests on the second segment of the metathoracic tarsus of *C. pegala* gave inconclusive results, while tests on this segment of *L. carydice* gave definitely negative results. A total of 51 paired tests ( $H_2O$  vs. sucrose solution) were made in 10 days.

*Ctenucha virginica* proved to be an easy animal to study. A total of about 250 paired tests were made in 10 days. All the experimental methods were used. The palpi lack contact chemoreceptors mediating proboscis extension and the antennae seemed to lack them in the early tests. All the tarsi possess the organs. On the prothoracic and mesothoracic tarsi they are on the ventral sides of all five of the tarsal segments, but not on the distal parts of the tibiae. On the metathoracic tarsi, the receptors are on the ventral sides of the three terminal segments, probably on the second segment, and probably not on the basal segment and the tibia. The distal  $\frac{1}{4}$  to  $\frac{1}{5}$ , only, of the proboscis proved to be sensitive when tested with glass microneedles bearing 1 *M* sucrose solution after control tests with water. When the tarsi or the tip of the proboscis was touched with a 1 *N* NaCl solution, the animal drew the proboscis into a tighter coil. If the animal was feeding on sucrose solution, touching the tip of the proboscis with NaCl solution caused immediate recoiling of the proboscis. Thus these receptors allow differentiation between acceptable and unacceptable solutions.

*Scepsis fulvicollis* also proved to be easy to test, and all the methods for locating the receptors were used. A total of about 400 pairs of tests were made in 16 days. Two additional methods were also used with these. First, the insects were tested with saturated sucrose solution as well as 1 *M* solution. Second, after tests on the legs had been made, all the legs were removed and the antennae and palpi tested. In all the previous tests, the antennae and palpi were tested with the animals intact or with only the prothoracic or prothoracic and mesothoracic legs removed.

The palpi seem not to bear receptors mediating the proboscis response. The ventral surfaces of the three pairs of tarsi bear end-organs. On the fore-leg, the receptors are on all five segments of the tarsus, but not on the tibia. On the mid-leg, the distal four segments of the tarsus definitely bear receptors. When tested with 1 *M* sucrose solution, the basal segment seemed not to bear the receptors, but when tested with saturated sucrose solution definite positive responses were obtained. The tibia seemed not to have receptors. The hind-leg showed a similar situation. When tested with 1 *M* sucrose solution, only the three distal segments of the tarsus seemed to have receptors, but when tested with saturated sucrose solution all five of the segments proved to bear them. The tibia again seemed not to have these receptors. The distal  $\frac{1}{5}$  to  $\frac{1}{6}$  of the proboscis also proved sensitive to

sucrose solutions; uncoiling was elicited on contact with a needle moistened with the sucrose solutions. The other parts of the proboscis could be touched with sucrose solutions without eliciting uncoiling. NaCl solution, as in *Ctenucha*, elicited a rejection reaction.

The situation with respect to the antennae in *Scepsis* was interesting. As long as the animals had tarsi, the antennae seemed to lack the receptors. Only occasionally would the insect uncoil the proboscis when sugar-water touched the antennae, and usually the insect would then take water and afterward be negative to sugar-water. If only the hind tarsi or even one hind tarsus alone were present, the reaction to antennal contact indicated lack of receptors. When all the legs were removed, however, the antennae seemed receptive, and the differential response to



FIGURE 1. Terminal portion of proboscis of *Ctenucha virginica* ( $\times 70$ ).

sucrose solution became almost perfect. By the time that this discovery was made, the individuals of the other species had died, and it was impossible to test them similarly.

The previous experiments were made during the summer of 1955. In January, 1956, adults of *Ctenucha virginica* were obtained from caterpillars reared from eggs laid during the past summer. Six of the adults (3 ♂♂ and 3 ♀♀) were tested and found to have antennal contact chemoreceptors. When either or both of the first two pairs of legs were present, no consistent reactions were obtained on stimulation of the antennae with sucrose solutions. When the first two pairs of legs were removed, leaving only the metathoracic legs, stimulation of the antennae with sucrose solutions consistently evoked the feeding response. Smyth (personal



FIGURE 2. Section of terminal portion of proboscis of *Ctenucha virginica* ( $\times 350$ ) to show probable chemoreceptive sensilla. The convex side has a rather dense layer of non-sensory epicuticular "hairs."



FIGURE 3. Terminal portion of proboscis of *Scepsis fulvicollis* ( $\times 70$ ).



communication) has recently recorded discharges from receptors on the antenna of *Scepsis* when they were stimulated with sucrose solutions, thus confirming electrophysiologically the results of the behavioral experiments.

Morphological studies on the tarsi of these Lepidoptera have not enabled us to select any particular sensilla as the end-organs. There are present, as in *Danaus plexippus* (Frings and Frings, 1949), short, thin-walled trichoid sensilla on the ventral surfaces of the tarsi among other stouter hairs and spines. These could be the receptors, but there is no proof of this.

On the proboscis of the moths studied, at the terminal portion found by experiments to be sensitive, there are many large sensilla (Figs. 1-4) that are almost certainly the contact chemoreceptors. These have a peculiar structure, appearing something like candles. There is a shaft, which is polygonal in cross-section, 6-7-



FIGURE 4. Section of terminal portion of proboscis of *Scepsis fulvicollis* ( $\times 350$ ) to show probable chemoreceptive sensilla. The epicuticular "hairs" on the convex surface are shorter than in *Ctenucha*.

sided in *Ctenucha* and 5-7-sided in *Scepsis*, with the angles seemingly thickened. This is capped with a thin-walled nipple-shaped structure, around the base of which the thickened corners of the shaft extend as points. In *Ctenucha* these range in size from 45-50  $\mu$  long and 12  $\mu$  thick at the proximal end of the group to 35-40  $\mu$  long and 12  $\mu$  thick near the tip, most being 40-45  $\mu$  long and 12  $\mu$  thick. At the tip there is a small group of shorter organs 20-25  $\mu$  long and 12  $\mu$  thick. In *Scepsis* the organs are somewhat smaller: 40-45  $\mu$  long and 9  $\mu$  thick at the proximal end decreasing regularly in size toward the tip to 25-30  $\mu$  long and 7  $\mu$  thick, most being 30-40  $\mu$  long and 7  $\mu$  thick. The organs in the group at the tip measure 15-18  $\mu$  long and 7  $\mu$  thick. The exact designation that one should give to these sensilla is not clear. Are they modified trichoids or basiconics? Since no other obvious sensilla appear at this region of the proboscis and these correspond in distribution

with the experimentally determined sensitive region, they are probably the end-organs.

Among the butterflies, we have examined only the proboscis of *C. pegala*. The distal  $\frac{1}{5}$  of it bears similar organs, 30–35  $\mu$  long and 7  $\mu$  thick, varying little in size throughout. These correspond in position also with the experimentally determined contact chemoreceptors and are thus probably the end-organs.

#### DISCUSSION

The results of this study and those of previous works (Minnich, 1921, 1922a, 1922b; Anderson, 1932; Weis, 1930; Frings and Frings, 1949) indicate that the butterflies (Superfamily: Papilionoidea) generally have contact chemoreceptors sensitive to sucrose and mediating feeding responses (trophic contact chemoreceptors) on the tarsi and distal part of the proboscis. The following species have been shown experimentally to possess them:

Family: Satyridae

*Lethe eurydice*

*Cercyonis pegala*

Family: Danaidae

*Danaus plexippus*

Family: Nymphalidae

*Speyeria cybele*

*Limnitis arthemis*

Family: Pieridae

*Colias philodice*

*Pieris rapae*

The following species have been shown to have contact chemoreceptors on the tarsi and almost certainly on the proboscis, but the latter has not yet been shown experimentally.

Family: Lycaenidae

*Lycaena thoe*

Family: Nymphalidae

*Phyciodes tharos*

*Nymphalis antiopa*

*Vanessa atalanta*

*Limnitis archippus*

Family: Papilionidae

*Papilio machaon*

*Papilio polyxenes*

In the Superfamily Hesperioidea (Skippers), *Polites mystic* has been reported by Anderson (1932) as showing only questionable discrimination by the tarsi between sucrose solutions and water. The proboscis was not tested. From preliminary observations on eight individuals (*Hesperia* or *Polites* spp.) it seems highly probable to us that tarsal and proboscidal receptors are present, but more

critical tests are needed. These Lepidoptera, as noted by Anderson, are erratic in response, often going for three or four days without taking water or sucrose solutions.

Among moths, Weis (1930) has reported that the following species lack tarsal chemoreceptors; other parts were untested.

Family: Sphingidae

*Macroglossa stellatarum*

*Acherontia atropos*

*Sphinx pinastri*

Family: Saturniidae

*Aglia tau*

Our results show that the following have tarsal, proboscidal and antennal receptors.

Family: Amatidae

*Ctenucha virginica*

*Scopsis fulvicollis*

The situation in moths needs much more study with many more species from a number of families. Many species of moths are known to have rudimentary mouthparts and to pass the adult life without feeding; it seems hardly likely that they would have contact chemoreceptors. The habits of the sphingid moths would seem to limit the utility of tarsal chemoreceptors. The day-flying, flower-feeding habits of the Amatidae make their sensory requirements like those of butterflies. A broader study might indicate how far this seeming adaptation of chemoreceptors to habits is found.

The results with the antennae of *Scopsis* and *Ctenucha* clearly mean that further testing is necessary before it is safe to conclude that antennal receptors are not present in other Lepidoptera. It is obvious that the conditions under which tests of contact chemoreception are made must be carefully specified. It seemed earlier (Frings and Frings, 1949) that antennal contact chemoreceptors were present only in Hymenoptera. Since then, Roth and Willis (1952) have reported them in cockroaches. They used sexual reactions rather than feeding reactions. The change in behavioral index of reception thus changed the interpretation of the location of the receptors. With the legs present, *Scopsis* and *Ctenucha* seemed to lack antennal trophic contact chemoreceptors. With the legs removed, they seemed to be present. With 1 M sucrose solution, *Scopsis* seemed to have receptors only on the distal segments of the mesothoracic and metathoracic tarsi. But with saturated sucrose solution, all segments were clearly sensitive. Regardless of theoretical interpretations one wishes to make of these cases, the necessity for exact specification of conditions of testing is obvious. All the receptors available to the animals may not be used in any specific behavioral situation (Tinbergen, 1951, pp. 25-37), or the receptors used in a particular behavior pattern may have different thresholds. Further, we may conclude that, while positive reactions in tests such as these indicate the presence of contact chemoreceptors, negative reactions do not necessarily show their absence. Negative results must be interpreted with great caution.



## SUMMARY

1. The loci of contact chemoreceptors, stimulation of which by sucrose solutions elicits proboscis extension, were determined for the butterflies: *Cercyonis pegala* and *Lethe eurydice* of the Satyridae, and *Speyeria cybele* and *Limnitis arthemis* of the Nymphalidae; and the diurnal moths: *Ctenucha virginica* and *Scepsis fulvicollis* of the Amatidae.

2. Local contact and excision methods were used. All have the receptors on the terminal part of the proboscis and on all the functional tarsi. The palpi probably do not bear the receptors, and in the butterflies the antennae likewise. In *Ctenucha* removal of the first two pairs of legs and in *Scepsis* removal of all the legs resulted in feeding reactions on stimulation of the antennae with sucrose solutions.

3. These results emphasize the need for careful control and specification of testing conditions and for cautious interpretation of negative results in experiments such as these.

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